BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 21/00, C12N 15/11, 9/10, C12Q 1/68 | A2

(11) International Publication Number: WO 97/44346

(43) International Publication Date: 27 November 1997 (27.11.97)

(21) International Application Number: PCT/IB97/00879

(22) International Filing Date: 22 May 1997 (22.05.97)

(30) Priority Data:
08/653,954 22 May 1996 (22.05.96) US

(71) Applicant *(for all designated States except US)*: McGILL UNIVERSITY [CA/CA]; 845 Sherbrooke Street West, Montréal, Québec H3A 1B1 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SZYF, Moshe [CA/CA]; 5770 Melling, Cote St. Luc, Québec H4W 2Z7 (CA). BIGEY, Pascal [FR/FR]; 3, rue Docteur Chibert, F-63000 Clermont-Ferrand (FR).

(74) Agent: CÔTÉ, France; Swabey Ogilvy Renault, Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: SPECIFIC INHIBITORS OF DNA METHYLTRANSFERASE ENZYME

(57) Abstract

The invention provides novel mechanism-based inhibitors of DNA methyltransferase enzyme, and diagnostic and therapeutic uses for the same. The novel inhibitors according to the invention form stable, noncovalent complexes with DNA methyltransferase enzyme in a manner which is independent of S-adenosylmethionine.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

SPECIFIC INHIBITORS OF DNA METHYLTRANSFERASE ENZYME

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to modulation of gene expression. In particular, the invention relates to modulation of gene expression of the gene encoding DNA methyltransferase, and to modulation of gene expression that is regulated by the enzyme DNA methyltransferase.

Summary of the Related Art

Modulation of gene expression has become an increasingly important approach to understanding various cellular processes and their underlying biochemical pathways. Such understanding enriches scientific knowledge and helps lead to new discoveries of how aberrancies in such pathways can lead to serious disease states. Ultimately, such discoveries can lead to the development of effective therapeutic treatments for these diseases.

One type of cellular process that is of particular interest is how the cell regulates the expression of its genes. Aberrant gene expression appears to be responsible for a wide variety of inherited genetic disorders, and has also been implicated in numerous cancers and other diseases. Regulation of gene expression is a complex process, and many aspects of this process remain to be understood. One of the mysteries of this process resides in the fact that while the genetic information is the same in all tissues that constitute a multicellular organism, the expression of functions encoded by the genome varies significantly in different tissues.

In some cases, tissue-specific transcription factors are known to play a role in this phenomenon. (See Maniatis *et al.*, Science 236: 1237-1245 (1987); Ingarham *et al.*, Annual Review of Physiology 52: 773-791 (1990). However, several important cases exist that cannot be readily explained by the action of transcription factors alone.

For example, Midgeon, Trends Genet. 10: 230-235 (1994), teaches that X-inactivation involves the inactivation of an allele of a gene that resides on the inactive X-chromosome, while the allele on the active X-chromosome continues to be expressed. In addition, Peterson and Sapienza, Annu. Rev. Genet. 27: 7-31 (1993), describes "parental imprinting", where an allele of a gene that is inherited from one parent is active and the other allele inherited from the other parent is inactive. In both of these cases, both alleles exist in an environment containing the same transcription factors, yet one allele is expressed and the other is silent. Thus, something other than transcription factors must be involved in these phenomena.

Investigators have been probing what type of "epigenetic information" may be involved in this additional control of the expression pattern of the genome. Holliday, Philos. Trans. R. Soc. Lond. B. Biol. Sci. 326: 329-338 (1990) discusses the possible role for DNA methylation in such epigenetic inheritance. DNA contains a set of modifications that is not encoded in the genetic sequence, but is added covalently to DNA using a different enzymatic machinery. These modifications take the form of methylation at the 5 position of cytosine bases in CG dinucleotides. Numerous studies have suggested that such methylation may well be involved in regulating gene expression, but its precise role has remained elusive. For example, Lock *et al.*, Cell 48: 39-46 (1987), raises questions about whether the timing of hypermethylation and X-inactivation is consistent with a causal role for methylation. Similarly, Bartolomei *et al.*, Genes Dev. 7: 1663-1673 (1993) and Brandeis *et al.*, EMBO J. 12: 3669-3677 (1993), disclose timing/ causation questions for the role of methylation in parental imprinting.

Some of the shortcomings of existing studies of the role of DNA methylation in gene expression reside in the

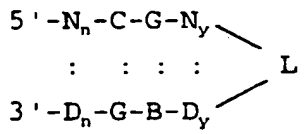
tools that are currently available for conducting the studies. Many studies have employed 5-azaC to inhibit DNA methylation. However, 5-azaC is a nucleoside analog that has multiple effects on cellular mechanisms other than DNA methylation, thus making it difficult to interpret data obtained from these studies. Similarly, 5-azadC forms a mechanism based inhibitor upon integration into DNA, but it can cause trapping of DNA methyltransferase (MTase) molecules on the DNA, resulting in toxicities that may obscure data interpretation.

More recently, Szyf *et al.*, J. Biol. Chem. 267: 12831-12836 (1995), discloses a more promising approach using expression of antisense RNA complementary to the DNA MTase gene to study the effect of methylation on cancer cells. Szyf and Von Hofe, PCT/US94/13685 (1994), discloses the use of antisense oligonucleotides complementary to the DNA MTase gene to inhibit tumorigenicity. These developments have provided powerful new tools for probing the role of methylation in numerous cellular processes. In addition, they have provided promising new approaches for developing therapeutic compounds that can modulate DNA methylation. One limitation to these approaches is that their effect is not immediate, due to the half life of DNA MTase enzyme. Thus, although the expression of DNA MTase is modulated, residual DNA MTase enzyme can continue to methylate DNA until such residual enzyme is degraded. There is, therefore, a need for new inhibitors of DNA MTase enzyme which are effective at inhibiting methylation, but without the toxic side effects of the earlier mechanism-based inhibitors.

BRIEF SUMMARY OF THE INVENTION

The invention provides novel inhibitors of DNA MTase enzyme and methods for using such inhibitors as analytical and diagnostic tools, as potentiators of transgenic plant and animal studies and gene therapy approaches, and as potential therapeutic agents.

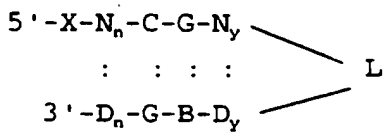
In a first aspect, the invention provides novel hairpin oligonucleotide inhibitors of DNA methyltransferase (DNA MTase) enzyme. The normal substrate for DNA MTase is a hemimethylated double stranded DNA molecule having a CG dinucleotide opposite a 5-methyl CG dinucleotide, *e.g.*, in a hairpin forming oligonucleotide. Methylation occurs at the 5-position of the cytosine base in the CG dinucleotide. The present inventors have discovered that substitution of the CG dinucleotide with an IG, UG, 5-bromocytosineG or 5-fluorocytosineG dinucleotide in a hairpin forming oligonucleotide results in a powerful mechanism-based inhibitor of DNA MTase. Thus, inhibitors according to this aspect of the invention have the general structure:



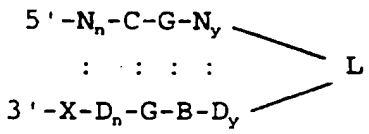
wherein each N is independently any nucleotide, n is a number from 0-20, C is 5-methylcytidine, G is guanidine, y is a number from 0-20, L is a linker, each D is a nucleotide that is complementary to an N such that Watson-Crick base pairing takes place between that D and the N such that the N_n-C-G-N_y and the D_n-G-B-D_y form a double helix, B is cytosine, inosine, uridine, 5-bromocytosine, or 5-fluorocytosine, dotted lines between nucleotides represent hydrogen bonding between the nucleotides, and the total number of nucleotides ranges from about 10 to about 50. In one preferred embodiment, L is an oligonucleotide

region having from 1 to about 10 nucleotides. DNA MTase inhibitors according to this aspect of the invention bind DNA MTase enzyme avidly in a noncovalent manner and inhibit DNA MTase in an S-adenosylmethionine (SAM)-independent manner.

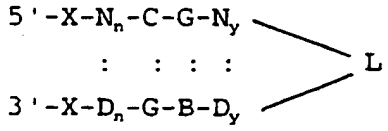
In a second aspect, the invention provides inhibitors of DNA MTase enzyme which also inhibit the expression of the DNA MTase gene. Inhibitors according to this aspect of the invention have the general structure:



or



or



wherein the substituents are the same as for inhibitors according to the first aspect of the invention, except that X is an antisense oligonucleotide of from about 10 to about 50 nucleotides in length, which is complementary to a portion of an RNA encoding DNA MTase enzyme, and L can optionally be X.

In a third aspect, the invention provides a diagnostic method for determining whether a particular sample of cells is cancerous. The method according to this aspect of the invention comprises preparing an extract from the cells in

the cell sample, adding labeled inhibitor according to the invention, measuring the extent of formation of a complex between the labeled inhibitor and DNA MTase enzyme, normalizing the level of such complex formation to the number of cells represented in the sample to obtain a normalized complex formation value, and comparing the normalized complex formation value to a normalized complex formation value for non-cancerous and/ or cancerous cell samples. In a preferred embodiment, the extract is a nuclear extract. Because cancer cells express DNA MTase at much higher levels than do non-cancerous cells, the comparison of the normalized complex formation values is diagnostic for whether the cell sample is cancerous.

In a fourth aspect, the invention provides methods for inhibiting tumorigenesis comprising administering to an animal, including a human, inhibitors according to the invention. In the method according to this aspect of the invention a therapeutically effective amount of an inhibitor according to the invention is administered for a therapeutically effective period of time to an animal, including a human, which has cancer cells present in its body.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagrammatic representation showing inhibition of DNA MTase enzyme by certain preferred embodiments of DNA MTase enzyme inhibitors according to the invention.

Figure 2 are representations of autoradiographs (panels A, B and D) and Western blots (panel C) in an experiment to identify complex formation between DNA MTase inhibitors and DNA MTase enzyme. Complex formation was reversed by boiling, and was independent of SAM.

Figure 3 are representations of Western blots showing that the complex formation between DNA MTase inhibitors and DNA MTase enzyme is completed within 30 minutes.

Figure 4: are representations of Western blots, panel A shows results of complex formation studies in which nuclear extracts were incubated with labeled oligonucleotide substrate (C) or inhibitor (I or U), followed by addition of a 100-fold excess of unlabeled substrate or inhibitor. Panel B shows results of complex formation studies in which nuclear extracts were incubated with 50 μM unlabeled oligonucleotide substrate (C), inhibitor (I or U), or with natural hemimethylated DNA substrate (HM) followed by addition of 0.5 μM labeled substrate or inhibitor.

Figure 5 is a representation of a blot showing time-dependent cellular uptake of a preferred embodiment of DNA MTase enzyme inhibitors according to the invention.

Figure 6 is a diagrammatic representation showing dose-dependent inhibition of soft agar colony formation by Y1 cells treated with antisense oligonucleotides complementary to DNA MTase coding sequence.

Figure 7 are diagrammatic representations showing reduction in tumor size in tumor bearing mice treated with antisense oligonucleotides complementary to DNA MTase coding sequence.

Figure 8 are representations of blots showing the intracellular localization in the nucleus of hemimethylated-hairpin substrate of DNA Mtase 1 hour post treatment (Panel A), 4 hours post treatment (Panel B), and 24 hours post treatment(Panel C).

Figure 9 is a diagrammatic representation showing inhibition of DNA MTase activity in cells treated with the hemimethylated test inhibitor having the sequence of SEQ. ID. NO. 13 at a concentration of 100 nM.

Figure 10 is a diagrammatic representation showing an overall reduction in the percentage of non-methylated CG dinucleotides in DNA MTase inhibitor-treated cells as compared to untreated cells.

Figure 11 is a diagrammatic representation showing a dose-dependent reduction in growth on soft agar observed following treatment with DNA MTase enzyme inhibitors according to the invention.

Figure 12 is a diagrammatic representation showing a dose-dependent reduction in cell number following treatment with DNA MTase enzyme inhibitors according to the invention.

Figure 13 is a diagrammatic representation of a gel shift assay showing the binding of human DNA MTase to the MTase enzyme inhibitors according to the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention relates to modulation of gene expression. In particular, the invention relates to modulation of gene expression of the gene encoding DNA methyltransferase, and to modulation of gene expression that is regulated by the enzyme DNA methyltransferase. The patents and publications identified in this specification are within the knowledge of those skilled in this field and are hereby incorporated by reference in their entirety.

The invention provides novel inhibitors of DNA MTase enzyme and methods for using such inhibitors as analytical and diagnostic tools, as potentiators of transgenic plant and animal studies and gene therapy approaches, and as potential therapeutic agents.

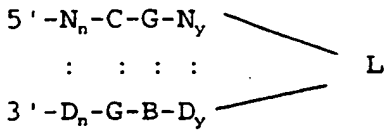
In a first aspect, the invention provides novel hairpin oligonucleotide inhibitors of DNA methyltransferase (DNA MTase) enzyme.

As used herein, "DNA methyltransferase" is a protein which is capable of methylating a particular DNA sequence.

In a preferred embodiment, the normal substrate for DNA MTase is a hemimethylated double stranded DNA molecule having a CG dinucleotide opposite a 5-methyl CG dinucleotide, *e.g.*, in a hairpin forming oligonucleotide, and methylation occurs at the 5-position of the cytosine base in the CG dinucleotide. Most preferably, the DNA methyltransferase is mammalian DNA methyltransferase or *M.SssI* DNA methyltransferase.

The present inventors have discovered that substitution of the CG dinucleotide with an IG, UG, 5-bromocytosineG, or 5-fluorocytosineG dinucleotide in a hairpin forming oligonucleotide results in a powerful mechanism-based inhibitor of DNA MTase. Thus, inhibitors having the general structure:

10



wherein each N is independently any nucleotide, n is a number from 0-20, C is 5-methylcytidine, G is guanidine, y is a number from 0-20, L is a linker, each D is a nucleotide that is complementary to an N such that Watson-Crick base pairing takes place between that D and the N such that the N_n-C-G-N_y and the D_n-G-B-D_y form a double helix, B is cytosine, inosine, uridine, 5-bromocytosine or 5-fluorocytosine, dotted lines between nucleotides represent hydrogen bonding between the nucleotides, and the total number of nucleotides ranges from about 10 to about 50. In one particularly preferred embodiment, L is a nucleoside or an oligonucleotide region having from 2 to about 10 nucleotides. Preferably, the indicated CG and GB and about 2 flanking nucleotides on either side are deoxyribonucleosides. DNA MTase inhibitors according to this aspect of the invention bind DNA MTase enzyme avidly in a noncovalent manner and inhibit DNA MTase in an S-adenosylmethionine (SAM)-independent manner.

Examples of certain preferred DNA MTase inhibitors according to this aspect of the invention include those having the following nucleotide sequences:

```
          m   m
5'-CTGAACGGATCG-T\
   :::::::::::  T        SEQ. ID. NO. 1
3'-GACTTGUCTAGC-T'
```

```
          m   m
5'-CTGAACGGATCG-T\
   :::::::::::  T        SEQ. ID. NO. 2
3'-GACTTGICTAGC-T'
```

```
          m
5'-CTGAACGGATCG-T\
   :::::::::::  T        SEQ. ID. NO. 3
3'-GACTTGUCTAGC-T/
```

```
          m
5'-CTGAACGGATCG-T\
   :::::::::::  T        SEQ. ID. NO. 4
3'-GACTTGICTAGC-T/
```

```
   m   m
5'-CGAACG-T\
   ::::::  T             SEQ. ID. NO. 5
3'-GITTGC-T/
       m
```

```
   m   m
5'-CGAACG-T\
   ::::::  T             SEQ. ID. NO. 6
3'-GITTGC-T/
```

```
   m
5'-CGAACG-T\
   ::::::  T             SEQ. ID. NO. 7
3'-GITTGC-T/
```

```
   m   m
5'-CGAACG-T\
   ::::::  T             SEQ. ID. NO. 8
3'-GUTTGC-T/
       m
```

```
   m   m
5'-CGAACG-T\
   ::::::  T             SEQ. ID. NO. 9
3'-GUTTGC-T/
```

```
   m
5'-CGAACG-T\
   ::::::  T             SEQ. ID. NO. 10
3'-GUTTGC-T/
```

```
          m
5'-CTGAACGCG-A
       :::    A
    3'-GBG-A
```
SEQ. ID. NO. 11

```
          m    m
5'-CTGAACGGATCG-T
   :::::::::::  T
3'-GACTTGBCTAGB-T
```
SEQ. ID. NO. 12

```
          m    m
5'-CTGAACGGATCG-T
   :::::::::::  T
3'-GACTTGCCTAGC-T
```
SEQ. ID. NO. 13

```
          m    m
5'-CTGAACGGATCG-T
   :::::::::::  T
3'-GACTTGFCTAGF-T
```
SEQ. ID. NO. 14

```
          m
5'-CTGAACGGATTG-T
   ::::::::::  :  T
3'-GACTTGCCTAGC-T
```
SEQ. ID. NO. 15

```
          m    m
5'-CTGAACGGATCG-T
   :::::::::::  T
3'-GACTTGXCTAGC-T
```
SEQ. ID. NO. 16

```
     m   m    m
5'-TCGAACGGATCG-T
   :::::::::::  T
3'-AGCTTGICTAGI-T
```
SEQ. ID. NO. 17

```
     m   m    m
5'-TCGAACGGATCG-T
   :::::::::::  T
3'-AICTTGICTAGI-T
```
SEQ. ID. NO. 18

```
          m
5'-CTGAACGCGA
       :::
    3'-GBGAA
```
SEQ. ID. NO. 19

```
    m   m
5'-CGAACGT-T
   : ::::   T
3'-GCTTGCT-T
```

SEQ. ID. NO. 20

```
    m   m
5'-CGAACGT-T
   : ::::   T
3'-GITTGCT-T
```

SEQ. ID. NO. 21

```
    m   m
5'-CGAACGT-T
   : ::::   T
3'-GUTTGCT-T
```

SEQ. ID. NO. 22

```
    m   m
5'-CGAACGT-T
   : ::::   T
3'-GFTTGFT-T
```

SEQ. ID. NO. 23

```
    m   m
5'-CGAACGT-T
   : ::::   T
3'-GBTTGBT-T
```

SEQ. ID. NO. 24

```
                          m
5'-CATCTGCCATTCCCACTCTACGCGA
                          :::
                       3'-GCGAA
```

SEQ. ID. NO. 25

```
                          m
5'-CATCTGCCATTCCCACTCTACGCGA
                          :::
                       3'-GIGAA
```

SEQ. ID. NO. 26

```
                          m
5'-CATCTGCCATTCCCACTCTACGCGA
                          :::
                       3'-GUGAA
```

SEQ. ID. NO. 27

```
                          m
5'-CATCTGCCATTCCCACTCTACGCGA
                          :::
                       3'-GFGAA
```

SEQ. ID. NO. 28

```
                                    m
5'-CATCTGCCATTCCCACTCTACGCGA)
                              :::   )
                           3'-GBGAA)
```
SEQ. ID. NO. 29

```
                                    m
5'-CATCTGCCATTCCCACTCTACGCGA)
                              :::   )
                           3'-GIGAA)
```
SEQ. ID. NO. 30

```
                                m
5'-CTGAACGCGA)
                          :::   )
                       3'-GCGAA)
```
SEQ. ID. NO. 31

```
                                m
5'-CTGAACGCGA)
                          :::   )
                       3'-GIGAA)
```
SEQ. ID. NO. 32

```
                                m
5'-CTGAACGCGA)
                          :::   )
                       3'-GUGAA)
```
SEQ. ID. NO. 33

wherein C is cytidine, T is thymidine, A is adenosine, G is guanine, m is a methyl group at the 5-position of cytosine, B is cytosine inosine, uridine, 5-bromocytidine, or 5-fluorouridine, and F is 5-fluorocytosine.

From the foregoing, those skilled in the art will recognize that the overall sequence of the oligonucleotide inhibitor of DNA MTase is not critical, so long as it is capable of forming a hairpin oligonucleotide in which one strand has a 5-methylCG dinucleotide and the other strand has, opposite the 5-methylCG dinucleotide, an IG, UG, 5-bromocytosineG, or 5-fluorocytosineG dinucleotide. For

purposes of the invention, the term "oligonucleotide" includes polymers of two or more deoxyribonucleotide, ribonucleotide, or 2'-O-substituted ribonucleotide monomers, or any combination thereof. Such monomers may be coupled to each other by any of the numerous known internucleoside linkages. In certain preferred embodiments, these internucleoside linkages may be phosphodiester, phosphotriester, phosphorothioate, or phosphoramidate linkages, or combinations thereof. In a particularly preferred embodiment the monomers are coupled by one or more phosphorothioate linkages.

The term oligonucleotide also encompasses such polymers having chemically modified bases or sugars and/ or having additional substituents, including without limitation lipophilic groups, intercalating agents, diamines and adamantane. For purposes of the invention the term "2'-O-substituted" means substitution of the 2' position of the pentose moiety with an -O-lower alkyl group containing 1-6 saturated or unsaturated carbon atoms, or with an -O-aryl or allyl group having 2-6 carbon atoms, wherein such alkyl, aryl or allyl group may be unsubstituted or may be substituted, *e.g.*, with halo, hydroxy, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl, or amino groups; or such 2' substitution may be with a hydroxy group (to produce a ribonucleoside), an amino or a halo group, but not with a 2'-H group.

Inhibitors according to the invention may conveniently be synthesized on a suitable solid support using well known chemical approaches, including H-phosphonate chemistry, phosphoramidite chemistry, or a combination of H-phosphonate chemistry and phosphoramidite chemistry (*i.e.*, H-phosphonate chemistry for some cycles and phosphoramidite chemistry for other cycles). Suitable solid supports include any of the standard solid supports used for solid

phase oligonucleotide synthesis, such as controlled-pore glass (CPG). (See, *e.g.*, Pon, Methods in Molec. Biol. 20: 465 (1993)).

DNA MTase inhibitors according to the invention are useful for a variety of purposes. For example, they can be used as "probes" of the physiological function of DNA MTase by being used to inhibit the activity of DNA MTase in an experimental cell culture or animal system and to evaluate the effect of inhibiting such DNA MTase activity. This is accomplished by administering to a cell or an animal a DNA MTase inhibitor according to the invention and observing any phenotypic effects. In this use, DNA MTase inhibitors according to the invention are preferable to traditional "gene knockout" approaches because they are easier to use and can be used to inhibit DNA MTase activity at selected stages of development or differentiation. Thus, DNA MTase inhibitors according to the invention can serve as probes to test the role of DNA methylation in various stages of development.

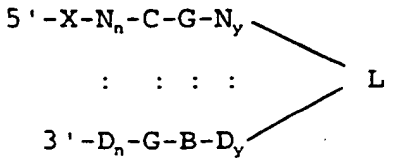
DNA MTase inhibitors according to the invention are useful as diagnostic probes for whether a cell sample is cancerous, as described in detail elsewhere in this specification. In addition, DNA MTase inhibitors according to the invention are useful for *in vivo* imaging of cancer cells. Since cancer cells have elevated levels of DNA MTase and normal cells do not, DNA MTase inhibitors according to the invention will form stable complexes with DNA MTase in cancer cells, but not in normal cells. Thus, appropriate labeling of DNA MTase inhibitors with an imaging agent, *e.g.*, technecium, will result in localization of the label at the site of the cancer cells. This effect may be enhanced by using DNA MTase inhibitors which are unstable (*e.g.*, oligonucleotide phosphodiesters) or rapidly cleared (*e.g.*, oligonucleotide

methylphosphonates) in the absence of complex formation, thus reducing background noise.

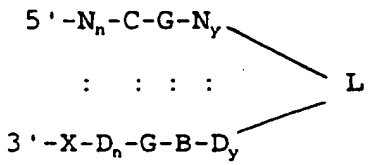
Finally, DNA MTase inhibitors according to the invention are useful in therapeutic approaches to cancer and other diseases involving suppression of gene expression. The anti-cancer utility of DNA MTase inhibitors according to the invention is described in detail elsewhere in this specification. In addition, DNA MTase inhibitors according to the invention may be used to activate silenced genes to provide a missing gene function and thus ameliorate disease symptoms. For example, the diseases beta thalassemia and sickle cell anemia are caused by aberrant expression of the adult beta globin gene. Most individuals suffering from these diseases have normal copies of the fetal gene for beta globin. However, the fetal gene is hypermethylated and is silent. Activation of the fetal globin gene could provide the needed globin function, thus ameliorating the disease symptoms.

For therapeutic use, DNA MTase inhibitors according to the invention may optionally be formulated with any of the well known pharmaceutically acceptable carriers or diluents. This formulation may further contain one or more additional DNA MTase inhibitors according to the invention. Alternatively, this formulation may contain one or more anti-DNA MTase antisense oligonucleotide or it may contain any other pharmacologically active agent.

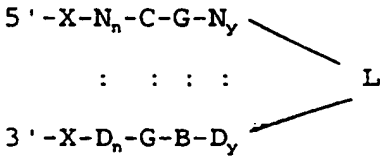
In a second aspect, the invention provides inhibitors of DNA MTase enzyme which also inhibit the expression of the DNA MTase gene. Inhibitors according to this aspect of the invention have the general structure:



or



or



wherein the substituents are the same as for inhibitors according to the first aspect of the invention, except that X is an antisense oligonucleotide of from about 10 to about 50 nucleotides in length, which is complementary to a portion of an RNA encoding DNA MTase enzyme, and L can optionally be X. Particularly preferred embodiments have the antisense oligonucleotide coupled at one or both ends to one or more of the inhibitors selected from the group consisting of SEQ. ID. NOS. 1-33.

In a third aspect, the invention provides a diagnostic method for determining whether a particular sample of cells is cancerous. The method according to this aspect of the invention comprises preparing an extract from the cells in the cell sample, adding labelled inhibitor according to the invention, measuring the extent of formation of a complex between the labeled inhibitor and DNA MTase enzyme, normalizing the extent of such complex formation to the number of cells represented in the sample to obtain a normalized complex formation value, and comparing the

normalized complex formation value to a normalized complex formation value for non-cancerous and/ or cancerous cell samples. In a preferred embodiment, the extract is a nuclear extract. Because cancer cells express DNA MTase at much higher levels than do non-cancerous cells, the comparison of the normalized complex formation values is diagnostic for whether the cell sample is cancerous.

In the diagnostic method according to this aspect of the invention, the extent of complex formation may be carried out in a variety of ways. For example, radiolabeled inhibitor may be used and the extent of incorporation of the inhibitor into a complex of appropriate size (*e.g.*, 190 kDa for a 27-mer inhibitor) can be determined. Alternatively, anti-DNA MTase antisera can be employed to determine the quantity of complex of appropriate size which is present. In another embodiment, antibodies or other binding partners can be prepared which recognize only the complex formed between the inhibitor and DNA MTase, and thus can be used to measure it formation. Normalizing the extent of complex formation to the number of cells can similarly be carried out in a variety of ways. For example, the number of cells in the test sample, as well as in the non-cancerous and cancerous control samples, can be counted prior to extract formation. Alternatively, the total amount of protein in each of the extracts can be determined using standard procedures. The normalized complex formation value can then be determined by dividing the extent of complex formation by the number of cells in the sample or the amount of protein in the extract.

In a fourth aspect, the invention provides methods for inhibiting tumorigenesis comprising administering to an animal, including a human, inhibitors according to the invention. In the method according to this aspect of the invention a therapeutically effective amount of a DNA MTase inhibitor according to the invention is administered for a

therapeutically effective period of time to an animal, including a human, which has cancer cells present in its body. Preferably, such administration should be parenteral, oral, sublingual, transdermal, topical, intranasal or intrarectal. Administration of the therapeutic compositions can be carried out using known procedures at dosages and for periods of time effective to reduce symptoms or surrogate markers of the cancer. When administered systemically, the therapeutic composition is preferably administered at a sufficient dosage to attain a blood level of DNA MTase inhibitor from about 0.01 micromolar to about 10 micromolar. For localized administration, much lower concentrations than this may be effective, and much higher concentrations may be tolerated. Preferably, a total dosage of DNA methyltransferase inhibitor will range from about 0.1 mg oligonucleotide per patient per day to about 200 mg oligonucleotide per kg body weight per day. It may desirable to administer simultaneously, or sequentially a therapeutically effective amount of one or more of the therapeutic compositions of the invention to an individual as a single treatment episode.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature.

Example 1

Inhibition of DNA MTase Activity in Nuclear Extracts Prepared from Human or Murine Cells

Nuclear extracts were prepared from 1 x 10^8 mid-log phase human H446 cells, human A549 cells or mouse Y1 cells. The cells were harvested and washed twice with phosphate buffered saline (PBS), then the cell pellet was resuspended in 0.5 ml Buffer A (10 mM Tris pH 8.0, 1.5 mM $MgCl_2$, 5 mM KCl_2, 0.5 mM DTT, 0.5 mM PMSF and 0.5% Nonidet P40) to

separate the nuclei from other cell components. The nuclei were pelleted by centrifugation in an eppendorf microfuge at 2,000 rpm for 15 min at 4°C. The nuclei were washed once in Buffer A and repelletted, then resuspended in 0.5 ml Buffer B (20 mM Tris pH 8.0, 0.25% glycerol, 1.5 mM $MgCl_2$, 0.5 mM PMSF, 0.2 mM EDTA 0.5 mM DTT and 0.4 mM NaCl). The resuspended nuclei were incubated on ice for 15 minutes then spun at 15,000 rpm to pellet nuclear debris. The nuclear extract in the supernatant was separated from the pellet and used for assays for DNA MTase activity. For each assay, carried out in triplicate, 3 micrograms of nuclear extract was used in a reaction mixture containing 0.1 micrograms of a synthetic 33-base pair hemimethylated DNA molecule substrate with 0.5 μCi S-[methyl-^{3}H] adenosyl-L-methionine (78.9 Ci/mmol) as the methyl donor in a buffer containing 20 mM Tris HCl (pH 7.4), 10 mM EDTA, 25% glycerol, 0.2 mM PMSF, and 20 mM 2-mercaptoethanol. The reaction mixture was incubated for 1 hour at 37°C to measure the initial rate of the DNA MTase. The reaction was stopped by adding 10% TCA to precipitate the DNA, then the samples were incubated at 4°C for 1 hour and the TCA precipitates were washed through GFC filters (Fischer). Controls were DNA incubated in the reaction mixture in the absence of nuclear extract, and nuclear extract incubated in the reaction mixture in the absence of DNA. The filters were laid in scintillation vials containing 5 ml scintillation cocktail and tritiated methyl groups incorporated into the DNA were counted in a beta scintillation counter. To measure inhibition of DNA MTase activity by different inhibitors, parallel reactions were carried out in which the inhibitors were added to the reaction mixtures in increasing concentrations ranging from 1 to 1000 nM. The control inhibitors had the same nucleotide sequence as the test inhibitors, except that the control inhibitor had an o-methyl modified ribose, or was a

scrambled oligonucleotide, whereas the test inhibitors had either an IG or UG dinucleotide, or a ^{5}-bromocytosine G, or a 5-fluorocytosine G dinucleotide or a cytosine opposite the 5-methylCG dinucleotide. The EC_{50} was calculated as the concentration of inhibitor required to inhibit 50% of the DNA MTase activity present in the nuclear extract.

Test inhibitors showed an EC_{50} of less than 1 μM with some embodiments showing an EC_{50} of as low as 30 nM. See Figure 1. The control inhibitor could not produce an EC_{50} at any concentration tested (up to 1 μM). Representative data using the test inhibitors of the invention are shown in table 1.

TABLE 1

TEST INHIBITOR	EC_{50}	SEQ. ID. NO.
m m 5'-CTGAACGGATCG-T\ :::::::::::: T 3'-GACTTGUCTAGC-T'	30 nM	1
m m 5'-CTGAACGGATCG-T\ :::::::::::: T 3'-GACTTGICTAGC-T'	30 nM	2
m 5'-CTGAACGGATCG-T\ :::::::::::: T 3'-GACTTGICTAGC-T'	350 nM	4
m m 5'-CTGAACGGATCG-T\ :::::::::::: T 3'-GACTTGCCTAGC-T'	30 nM	13
m m 5'-CTGAACGGATCG-T\ :::::::::::: T 3'-GACTTGFCTAGF-T'	50 nM	14
m 5'-CTGAACGGATTG-T\ :::::::::: : T 3'-GACTTGCCTAGC-T'	50 nM	15
m m 5'-CTGAACGGATCG-T\ :::::::::::: T 3'-GACTTGXCTAGC-T'	350 nM	16

TABLE 1 (cont'd)

TEST INHIBITOR	EC_{50}	SEQ. ID. NO.
m m m 5'-TCGAACGGATCG-T\ : : : : : : : : : : : : T 3'-AGCTTGICTAGI-T'	350 nM	17
m m m 5'-TCGAACGGATCG-T\ : : : : : : : : : : : : T 3'-AICTTGICTAGI-T'	450 nM	18
m 5'-CTGAACGCGA) : : : 3'-GBGAA)	450 nM	19
m m 5'-CGAACGT-T\ : : : : : T 3'-GCTTGCT-T/	500 nM	20
m m 5'-CGAACGT-T\ : : : : : T 3'-GITTGCT-T/	500 nM	21
m m 5'-CGAACGT-T\ : : : : : T 3'-GUTTGCT-T/	500 nM	22
m m 5'-CGAACGT-T\ : : : : : T 3'-GFTTGFT-T/	230 nM	23
m m 5'-CGAACGT-T\ : : : : : T 3'-GBTTGBT-T/	230 nM	24
m 5'-CATCTGCCATTCCCACTCTACGCGA) : : : 3'-GCGAA)	300 nM	25
m 5'-CATCTGCCATTCCCACTCTACGCGA) : : : 3'-GIGAA)	300 nM	26

5'-CATCTGCCATTCCCACTCTACGCGA (m) / 3'-GUGAA	300 nM	27
5'-CATCTGCCATTCCCACTCTACGCGA (m) / 3'-GFGAA	300 nM	28
5'-CATCTGCCATTCCCACTCTACGCGA (m) / 3'-GBGAA	50 nM	29
5'-CATCTGCCATTCCCACTCTACGCGA (m) / 3'-GIGAA	500 nM	30
5'-CTGAACGCGA (m) / 3'-GCGAA	700 nM	31
5'-CTGAACGCGA (m) / 3'-GIGAA	600nM	32
5'-CTGAACGCGA (m) / 3'-GUGAA	600nM	33

These results demonstrate that substitution of an IG or UG dinucleotide, or a 5-bromocytosine G, or a 5-fluorocytosine G dinucleotide opposite a 5-methylCG dinucleotide in a synthetic hairpin forming oligonucleotide results in an effective inhibitor of DNA MTase activity.

The results shown in Figure 9 demonstrate that the hemimethylated test inhibitor having the sequence of SEQ. ID. NO. 13 at a concentration of 100 nM inhibited DNA MTase activity as compared to the control nonmethylated hairpin.

Example 2

Complex Formation Between DNA MTase Inhibitors and DNA MTase Enzyme

To measure the rate of complex formation between different DNA MTase inhibitors and DNA MTase enzyme, different inhibitors (each at 4 micromolar concentration) were labeled using polynucleotide kinase and gamma ^{32}P-ATP (300 mCi/mmol, 50 μCi) (New England Biolabs, Beverly, MA) as recommended by the manufacturer. Labeled oligonucleotide was separated from nonincorporated radioactivity by passing through a G-50 Sephadex spin column (Pharmacia, Uppsala, Sweden). Labeled inhibitors (500 nM) were incubated with 5 micrograms nuclear extract prepared as described in Example 1. The incubation, in the same buffer used for the DNA MTase activity assay, was at 37°C for 30 minutes. To determine whether complex formation was dependent on the cofactor SAM, the reaction was carried out both in the presence and the absence of SAM). Then, loading dye (0.3 M Tris-HCl pH 8.8, 0.2% SDS, 10% glycerol, 28 mM 2-mercaptoethanol and 24 μg/ml bromophenol blue) was added and the sample was separated on a 5% SDS-polyacrylamide gel (SDS-PAGE) with a 4% stacking gel according to standard procedures. Following SDS-PAGE separation, the gel was exposed to autoradiography for visualization of a complex migrating at 190 kDa. Alternatively, the gel was electrotransferred onto a PVDF membrane (Amersham Life Sciences, Buckinghamshire, England) using a BioRad (Hercules, CA) electrotransfer apparatus at 250 milliamperes for 2.5 hours in electrotransfer buffer (3.03 g/l Tris base, 14.4 g/l glycine, 1 g/l SDS, pH 8.3) for Western blotting with a DNA MTase-specific antisera. The membrane was blocked for 1 hour in a buffer containing 5 mM Tris base, 200 mM NaCl, 0.5% Tween-20 and 5% dry milk. Rabbit antisera was raised according to standard procedures against a peptide sequence found in the catalytic domain of

human and murine DNA MTase (amino acids GGPPCQGFSGMNRFNSRTY). The antisera was added to the membrane at a 1:200 dilution and incubated for 1 hour. The membrane was washed with the blocking buffer, then reacted with a 1:5000 dilution of goat anti-rabbit secondary antibody (Amersham) for an additional hour. The membrane was then washed for 10 minutes in blocking buffer, three times, and bands reacting with anti-DNA MTase antibody were visualized using an ECL detection kit (Amersham). Typical results are shown in Figures 2 and 3. The results shown in Figure 2 demonstrate that a 190 kDa complex is detected by both autoradiography and Western blotting, strongly indicating that the 190 kDa complex is formed between the DNA MTase inhibitors and DNA MTase enzyme. These results further demonstrate that such complex formation is independent of the cofactor SAM. The results shown in Figure 3 demonstrate that the complex formation is complete within 30 minutes, thus suggesting that such complex formation provides an assay for the level of DNA MTase in different cell samples.

In addition, nuclear extracts prepared as described in Example 1, were incubated with labeled inhibitors to perform gel shift assays according to standard protocols (see e.g., *Molecular Cloning*, 2d Edition, Cold Spring Harbor Laboratory Press (1989)). The results shown in Figure 13, further demonstrate the binding of human DNA methyltransferase to the inhibitors of the invention.

Example 3

Stability of Noncovalent Complex Formation Between DNA MTase Inhibitors and DNA MTase Enzyme

To determine the stability of the complex between DNA MTase inhibitors and DNA MTase enzyme, relative to the stability of the complex between normal substrate and DNA MTase enzyme, binding competition assays were carried out

as follows. Complex formation was carried out as described in Example 2, except that the labeled substrate or inhibitor was allowed to form a complex with the DNA MTase, followed by addition of a 100-fold excess of unlabeled substrate or inhibitor. The substrate was a hairpin forming oligonucleotide having a 5-methylCG dinucleotide on one strand, opposite a CG dinucleotide on the other strand. The inhibitors were identical, except that they had an IG or UG dinucleotide on the other strand. The results are shown in Figure 4, panel A. Where complex formation was originally carried out using the substrate oligonucleotide, no radiolabeled complex was detected, suggesting that the complex is labile. However, where complex formation was originally carried out using either the IG or the UG inhibitor, the excess unlabeled substrate or inhibitor was unable to displace the radiolabel from the complex, indicating that the DNA MTase inhibitor-DNA MTase enzyme complex is very stable, with a slow off rate.

Alternatively, when 50 μM unlabeled substrate or inhibitor was pre-incubated with the nuclear extract, subsequent incubation with 0.5 μM radiolabeled substrate or inhibitor could not displace the unlabeled substrate or inhibitor from the complex. However, the labeled inhibitor could displace unlabeled hemi-methylated DNA, the natural substrate for DNA MTase, from such a pre-formed complex. These results demonstrate that binding of the inhibitors to DNA MTase enzyme is specific and saturable, and that such inhibitors are efficacious competitors of the natural substrate of DNA MTase (Figure 4, panel B).

Example 4

DNA MTase Inhibitor Accumulation and Formation of Complexes with DNA MTase Enzyme in Cells

DNA MTase inhibitors were labeled with ^{32}P as described in Example 2. 300,000 Y1 cells were plated per well in a

six well tissue culture plate. Labeled inhibitors were added to a final concentration of 1 micromolar. Cells were harvested at different time points by trypsinization and washed extensively with PBS to remove nonincorporated compounds. The cell pellet was resuspended in 20 μl buffer RIPA (0.5% deoxycholic acid, 0.1% SDS, 1% NP-40, in PBS). The homogenate was incubated at 4°C for 30 minutes, then spun in a microfuge at maximum speed for 30 minutes, after which the supernatant was transferred to a new tube. Two μl of supernatant were extracted with phenol-chloroform and loaded onto a 20% polyacrylamide-urea gel. Visualization was by autoradiography. The results demonstrated that the DNA MTase inhibitors were taken up by the cells in a time-dependent manner (Figure 5). Ten microliters of the supernatant are loaded directly on 5% SDS-PAGE and visualized by autoradiography to detect complex formation. It is expected that the 190 kDa complex between the 27-mer DNA MTase inhibitor and DNA MTase enzyme will be observed.

Example 5

Intracellular Localization of DNA MTase Inhibitors

100 nM of oligonucleotide having the sequence shown as SEQ. ID. NO. 22 were labeled with the mixed isomeric *N*-hydroxysuccinimide esters of 5(6)-carboxyfluorescein (Molecular Probes, Eugene, OR) as described by Sinha and Striepeke in Oligonucleotides and Analogues: A Practical Approach (1991)(Eckstein, Ed.) Oxford University Press, NY) pp 185-210. In a typical reaction, 28 nmol of oligonucleotide was dissolved in 180 µl of 0.4 M $NaHCO_3/Na_2CO_3$ pH 9.0, 1 M N-dimethylformamide/water (3:2:1 v/v/v). This solution was diluted with an equal volume of water, and 1.5 mg of active ester of the fluorophore was added. The mixture was kept at room temperature in the dark for 17 h with gentle shaking and then diluted to 6 ml with water. Most of the excess dye

was removed by extraction of the aqueous solution with *n*-butanol followed by ethanol precipitation according to standard methods. Human lung carcinoma A549 cells were grown to 10-5 and incubated with the labeled inhibitor in the presence of 10ug/me lipofectin. The subcellular localization of the labeled inhibitor at different time points was determined by fluorescent microscopy. As shown in Figure 8, a diffuse pattern of distribution of the test inhibitor in the cytosol and in the nuclei was observed 1 hour post treatment. At 4 hours post treatment almost all the test inhibitor was localized in the nucleus the site of action of DNA MTase. At 24 hours, the test inhibitor was mainly in the nucleus distributed in a punctate manner which is similar to the pattern of localization of DNA MTase. This experiment demonstrates that the DNA inhibitor is localized in the site of action of DNA MTase.

Example 6

Analysis of Cellular DNA Methylation in Cells Treated with DNA MTase Inhibitors

Nuclear extracts were prepared from untreated cells and from DNA MTase inhibitor-treated cells (1 μM inhibitor having SEQ ID NO: 13) according to the methodology described in Example 1. The DNA pellet was resuspended in 0.5 ml DNA extraction buffer (0.15 M NaCl, 1% SDS, 20 mM Tris-HCl pH 8.0, 5 mM EDTA), then 100 μg protease K was added and the suspension was incubated at 50°C for 16 hours. The DNA was extracted in phenol-chloroform by adding 0.25 ml phenol and 0.25 ml chloroform. The suspension was mixed and the organic and aqueous phases were separated by centrifugation in a microfuge for 10 minutes at 15,000 rpm. One ml absolute ethanol was added to the aqueous phase and the DNA was precipitated by centrifugation in a microfuge for 15 minutes at 15,000 rpm. The DNA pellet was washed in 70% ethanol and repelleted by

centrifugation. The DNA was resuspended in 100 μl 20 mM Tris-HCl pH 8.0, 1 mM EDTA.

Two μg DNA were incubated at 37°C for 15 minutes with 0.1 unit of DNAase, 2.5 μl ^{32}P-alpha-dGTP (3000 Ci/mmol, Amersham) and then 2 units Kornberg DNA polymerase (Boehringer Mannheim, Mannheim, Germany) were added and the reaction mixture was incubated for an additional 25 minutes at 30°C. Fifty μl water was then added and nonincorporated radioactivity was removed by spinning through a Microspin S-300 HR column (Pharmacia). Labelled DNA(20 μl) was digested with 70 μg micrococcal nuclease (Pharmacia) in the manufacturer's recommended buffer for 10 hours at 37°C. Equal amounts of radioactivity were loaded onto TLC phosphocellulose plates (Merck, Darmstadt, Germany) and the 3' mononucleotides were separated by chromatography in one direction, in 66:33:1 isobutyric acid/H_2O/NH_4OH. The chromatograms were exposed to XAR film (Eastman Kodak, Rochester, NY) and the autoradiograms were scanned by laser densitometry (Scanalytics, CSPI, Billerica, MA). Spots corresponding to cytosine and 5-methylcytosine were quantified and the percentage of non-methylated CG dinucleotides was determined. The results shown in figure 10 demonstrate an overall reduction in the percentage of non-methylated CG dinucleotides in DNA MTase inhibitor-treated cells, relative to untreated cells.

To asses demethylation of specific genes, a procedure is carried out as generally described in J. Biol. Chem. 270: 12690-12696 (1995). Briefly, the genomic DNA (10 μg) is extracted and subjected to digestion by 25 units HindIII, followed by digestion by either 25 units MspI (CG methylation insensitive) or 25 units HpaII (CG methylation sensitive) for 8 hours at 37°C. The digested DNA is separated on a 1.5% agarose gel and subjected to Southern blotting and hybridization with specific probes. The results are expected to show that genes which are

ordinarily heavily methylated in the test cells become undermethylated, whereas the methylation levels for genes which are not ordinarily heavily methylated in the test cells are not significantly affected.

Example 7

Inhibition of Tumorigenesis By Inhibitors of DNA MTase

A549 cells were plated on a 6 well plate at a density of 80,000 cells/well. DNA MTase enzyme inhibitors (from about 10 to about 1000 nanomolar) or antisense oligonucleotide phosphorothioates complementary to the DNA MTase coding sequence (about 0.5 to 20 micromolar) were added to the cells. The cells were similarly treated daily for 3 days. Then, the cells were harvested and 3,000 live cells were plated in soft agar, as described in Freedman and Shin, Cell 3: 355-359 (1974). Two weeks after plating, the number of colonies formed in soft agar were scored by visual examination. In the case of antisense oligonucleotides, a dose-dependent reduction in the number of colonies was observed (Figure 6). The results shown in Figure 11 demonstrate a dose-dependent reduction in growth on soft agar observed following 3 days treatment with DNA MTase enzyme inhibitors. The results shown in Figure 12 demonstrate a dose-dependent reduction cell number treatment with DNA MTase enzyme inhibitors.

Alternatively, 6 to 8 week old LAF-1 mice (Jackson Labs, Bar Harbor, ME) are injected subcutaneously in the flank area with 2 x 10^6 Y1 cells. Three days later, the mice are injected with 1-5 mg/kg antisense oligonucleotide phosphorothioates complementary to DNA MTase coding sequence, or with 5 mg/kg DNA MTase enzyme inhibitor. This dosing is repeated every two days. After one month, the mice are sacrificed and the tumor size is determined. In the case of the antisense oligonucleotides, significant reduction in tumor size was observed, relative to controls

treated with a randomized or a reverse antisense sequence (Figure 7). Similar results are expected for the DNA MTase enzyme inhibitors.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: McGill University

(ii) TITLE OF INVENTION: SPECIFIC INHIBITORS OF DNA METHLYTRANSFERASE ENZYME

(iii) NUMBER OF SEQUENCES: 33

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: HALE AND DORR LLP
(B) STREET: 60 State Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: United States of America
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version#1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US97/
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Keown, Wayne A.
(B) REGISTRATION NUMBER: 33,923
(C) REFERENCE/DOCKET NUMBER: 106.101.120PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 526-6000
(B) TELEFAX: (617) 526-5000

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTGAACGGAT CGTTTCGATC UGTTCAG 27

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:2 only, N is used to describe a base I as defined in the specifications."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CTGAACGGAT CGTTTCGATC NGTTCAG 27

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTGAACGGAT CGTTTCGAAT CGTTCAG 27

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:4 only, N is used to describe a base I as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTGAACGGAT CGTTTCGATC NGTTCAG 27

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:5 only, N is used to describe a base I as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGAACGTTTC GTTNG 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:6 only, N is used to describe a base I as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGAACGTTTC GTTNG 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:7 only, N is used to describe a base I as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGAACGTTTC GTTNG 15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGAACGTTTC GTTUG 15

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGAACGTTTC GTTUG 15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGAACGTTTC GTTUG 15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:11 only, N is used to describe a base B as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTGAACGCGA AAGNG 15

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:12 only, N is used to describe a base B as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTGAACGGAT CGTTTNGATC NGTTCAG 27

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTGAACGGAT CGTTTCGATC CGTTCAG 27

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27

(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:14 only, N is used to describe a base F as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTGAACGGAT CGTTTNGATC NGTTCAG 27

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTGAACGGAT TGTTTCGATC CGTTCAG 27

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:16 only, N is used to describe a base X as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGAACGGAT CGTTTCGATC NGTTCAG 27

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:17 only, N is used to describe a base I as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TCGAACGGAT CGTTTNGATC NGTTCGA 27

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:18 only, N is used to describe a base I as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TCGAACGGAT CGTTTNGATC NGTTCNA 27

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:19 only, N is used to describe a base B as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTGAACGCGA AAGNG 15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGAACGTTTT CGTTCG 16

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:21 only, N is used to describe a base I as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CGAACGTTTT CGTTNG 16

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGAACGTTTT CGTTUG 16

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:23 only, N is used to describe a base F as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CGAACGTTTT TNGTTNG 17

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:24 only, N is used to describe a base B as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGAACGTTTT NGTTNG 16

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CATCTGCCAT TCCCACTCTA CGCGAAAGCG 30

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..30
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:26 only, N is used to describe a base I as described in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CATCTGCCAT TCCCACTCTA CGCGAAAGNG 30

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CATCTGCCAT TCCCACTCTA CGCGAAAGUG 30

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..31
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:28 only, N is used to describe a base F as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CATCTGCCAT TTCCCACTCT ACGCGAAAGN G 31

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..30
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:29 only, N is used to describe a base B as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CATCTGCCAT TCCCACTCTA CGCGAAAGNG 30

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..30
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:30 only, N is used to describe a base I as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CATCTGCCAT TCCCACTCTA CGCGAAAGNG 30

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CTGAACGCGA AAGCG 15

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /note= "With respect to SEQ ID NO:32 only, N is used to describe a base I as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CTGAACGCGA AAGNG
15

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

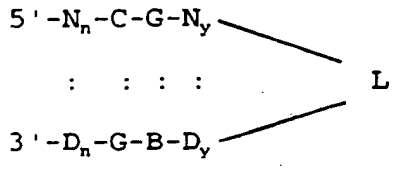
(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CTGAACGCGA AAGUG 15

What is claimed is:

1. An inhibitor of DNA methytransferase enzyme having the general structure:



wherein each N is independently any nucleotide, n is a number from 0-20, C is 5-methylcytidine, G is guanidine, y is a number from 0-20, L is a linker, each D is a nucleotide that is complementary to an N such that Watson-Crick base pairing takes place between that D and the N such that the N_n-C-G-N_y and the D_n-G-B-D_y form a double helix, B is cytosine, inosine, uridine, 5-bromocytosine or 5-fluorocytosine, dotted lines between nucleotides represent hydrogen bonding between the nucleotides, and the total number of nucleotides ranges from about 10 to about 50.

2. The inhibitor of DNA methytransferase enzyme according to claim 1, wherein the inhibitor is labeled.

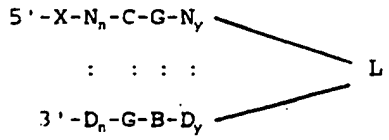
3. The inhibitor of DNA methytransferase enzyme according to claim 1, wherein L is a nucleoside or an oligonucleotide region having from 2 to about 10 nucleotides.

4. The inhibitor of DNA methytransferase enzyme according to claim 3 comprising at least one internucleoside linkage selected from the group consisting of phosphodiester, phosphotriester, phosphorothioate, or phosphoramidate linkages, or combinations thereof.

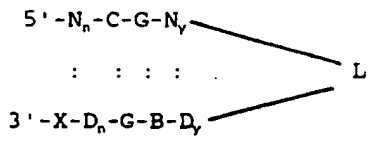
5. The inhibitor of DNA methytransferase enzyme according to claim 3 comprising at least one phosphorothioate internucleoside linkage.

6. The inhibitor of DNA methyltransferase enzyme according to claim 1, having the sequence selected from the group consisting of SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, SEQ. ID. NO. 5, SEQ. ID. NO. 6, SEQ. ID. NO. 7, SEQ. ID. NO. 8, SEQ. ID. NO. 9, SEQ. ID. NO. 10, SEQ. ID. NO. 11, SEQ. ID. NO. 12, SEQ. ID. NO. 13, SEQ. ID. NO. 14, SEQ. ID. NO. 15, SEQ. ID. NO. 16, SEQ. ID. NO. 17, SEQ. ID. NO. 18, SEQ. ID. NO. 19, SEQ. ID. NO. 20, SEQ. ID. NO. 21, SEQ. ID. NO. 22, SEQ. ID. NO. 23, SEQ. ID. NO. 24, SEQ. ID. NO. 25, SEQ. ID. NO. 26, SEQ. ID. NO. 27, SEQ. ID. NO. 28, SEQ. ID. NO. 29, SEQ. ID. NO. 30, SEQ. ID. NO. 31, SEQ. ID. NO. 32, and SEQ. ID. NO. 33.
wherein C is cytidine, T is thymidine, A is adenosine, G is guanine, m is a methyl group at the 5-position of cytosine, B is cytosine, inosine, uridine, 5-bromocytidine, or 5-fluorouridine, and F is 5-fluorocytosine.

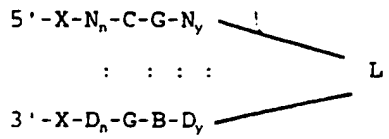
7. An inhibitor of DNA methytransferase enzyme having the general structure



or



or



wherein each N is independently any nucleotide, n is a number from 0-20, C is 5-methylcytidine, G is guanidine, y is a number from 0-20, L is a linker, each D is a nucleotide that is complementary to an N such that Watson-Crick base pairing takes place between that D and the N such that the N_n-C-G-N_y and the D_n-G-B-D_y form a double helix, B is cytosine, inosine, uridine, 5-bromocytosine, or 5-fluorocytosine, dotted lines between nucleotides represent hydrogen bonding between the nucleotides, and the total number of nucleotides ranges from about 10 to about 50, X is an antisense oligonucleotide of from about 10 to about 50 nucleotides in length, which is complementary to a portion of an RNA encoding DNA MTase enzyme, and L can optionally be X.

8. A diagnostic method for determining whether a particular sample of cells is cancerous, the method comprising preparing an extract from the cells in the cell sample, adding a labeled inhibitor according to claim 2, measuring the extent of formation of a complex between the labeled inhibitor and DNA MTase enzyme, normalizing the level of such complex formation to the number of cells represented in the sample to obtain a normalized complex formation value, and comparing the normalized complex formation value to a normalized complex formation value for non-cancerous and/ or cancerous cell samples.

9. The method according to claim 7, wherein the extract is a nuclear extract.

10. A method for inhibiting tumorigenesis in an animal, including a human, comprising administering to the animal, which has cancer cells present in its body, a therapeutically effective amount of an inhibitor according to claim 1 for a therapeutically effective period of time.

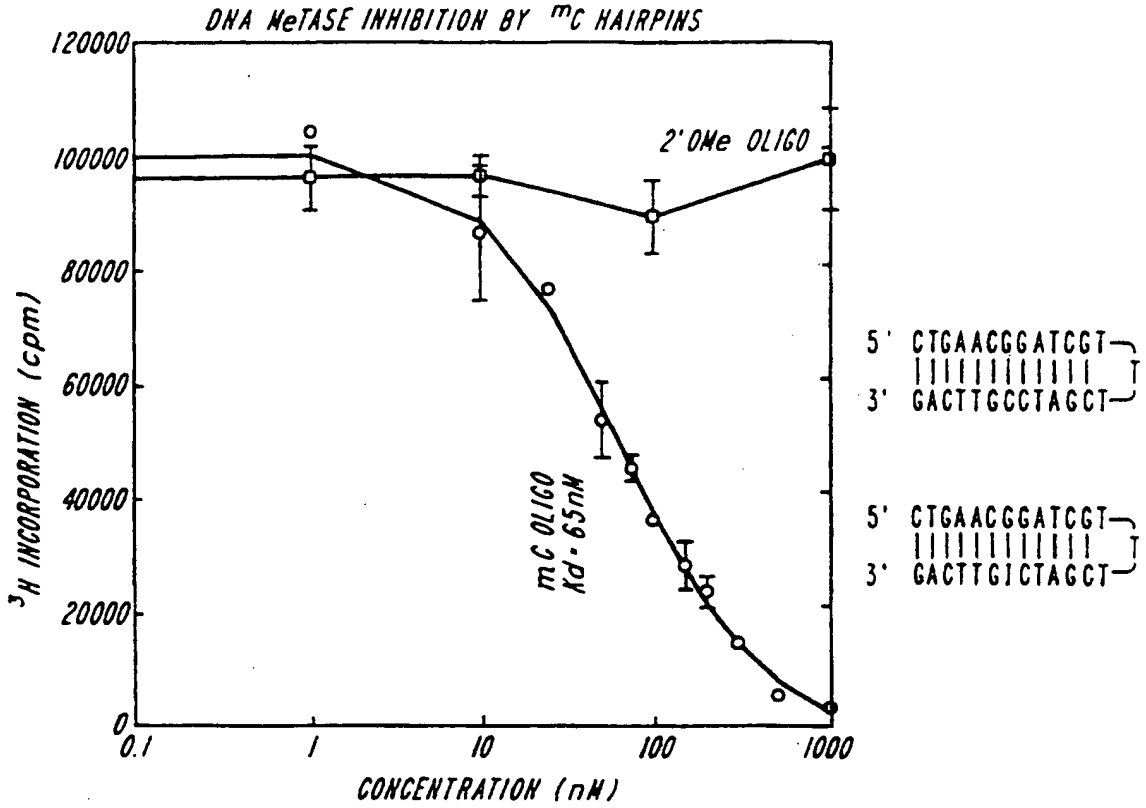


FIG. 1

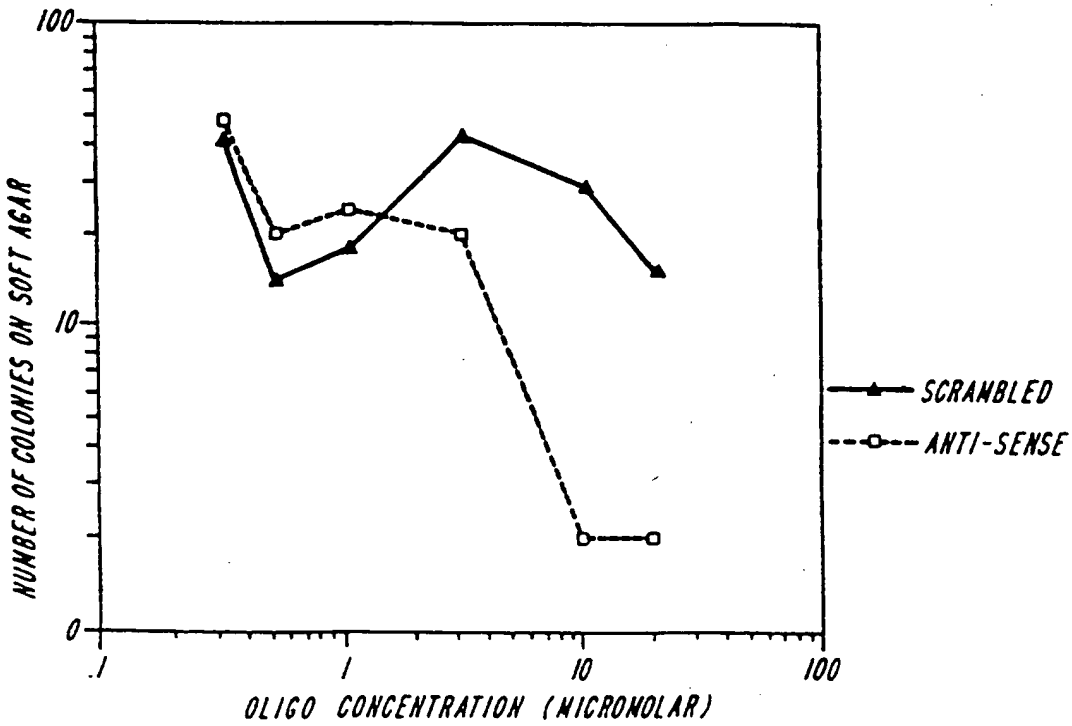


FIG. 6

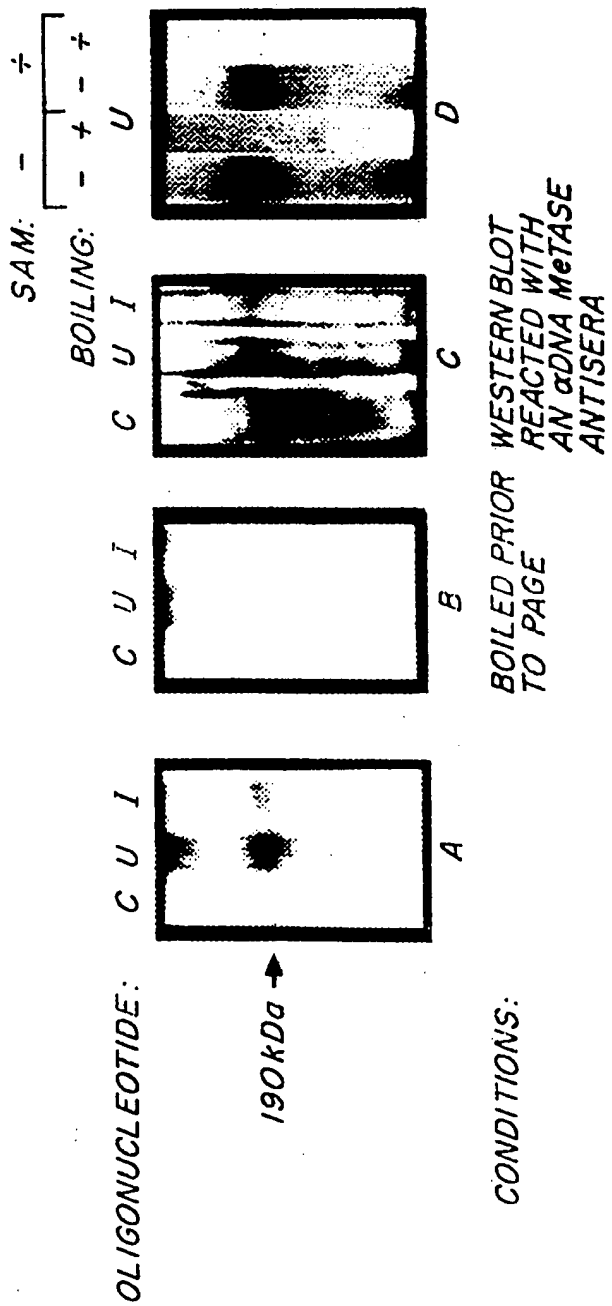

SAM: - +
BOILING: - + - +
OLIGONUCLEOTIDE: C U I C U I C U I U
190 kDa
A
B
C
D
CONDITIONS: BOILED PRIOR TO PAGE
WESTERN BLOT REACTED WITH AN αDNA MeTASE ANTISERA

FIG. 2

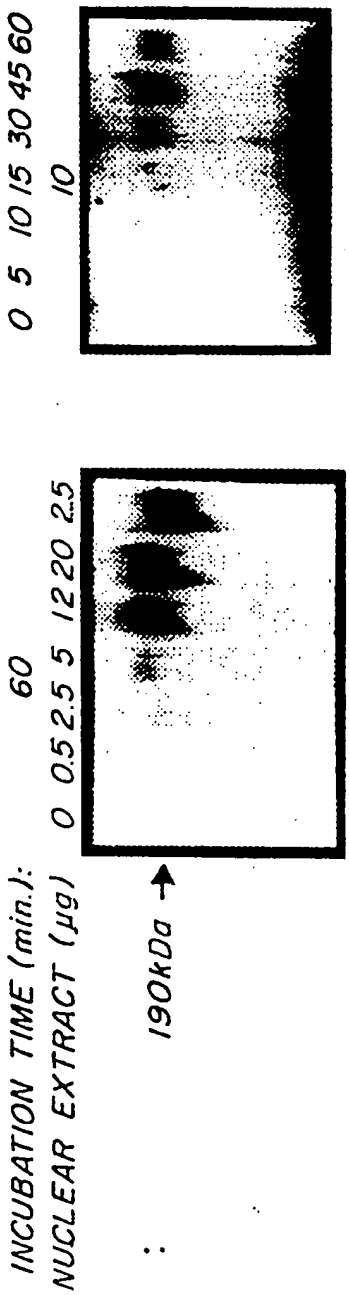


FIG. 3

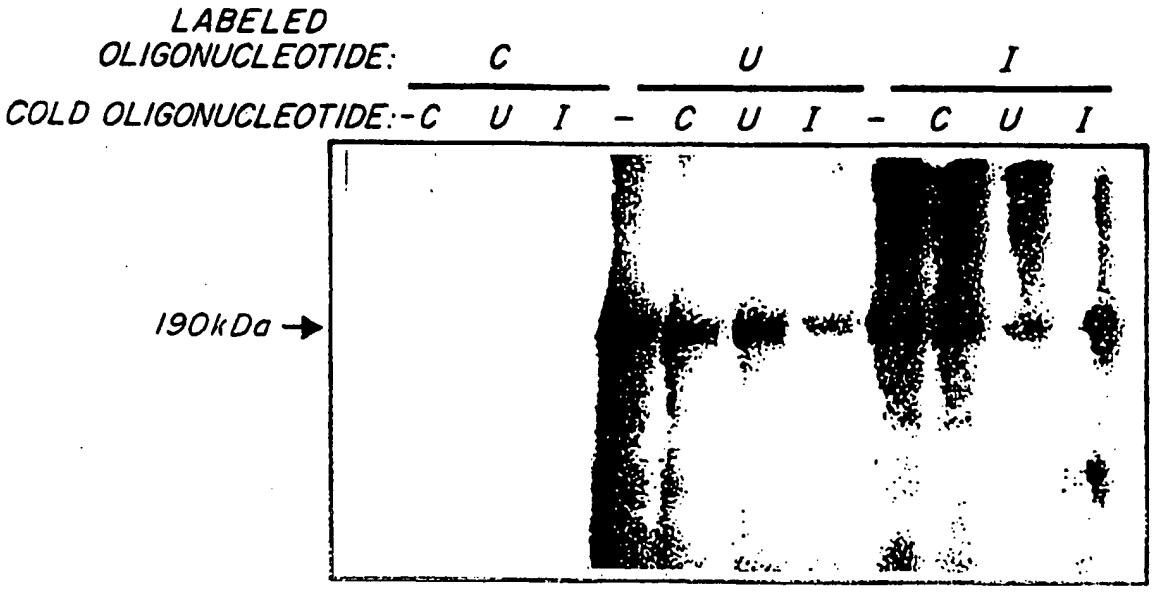

LABELED
OLIGONUCLEOTIDE:
C
U
I
COLD OLIGONUCLEOTIDE:-C U I - C U I - C U I
190kDa

FIG. 4A

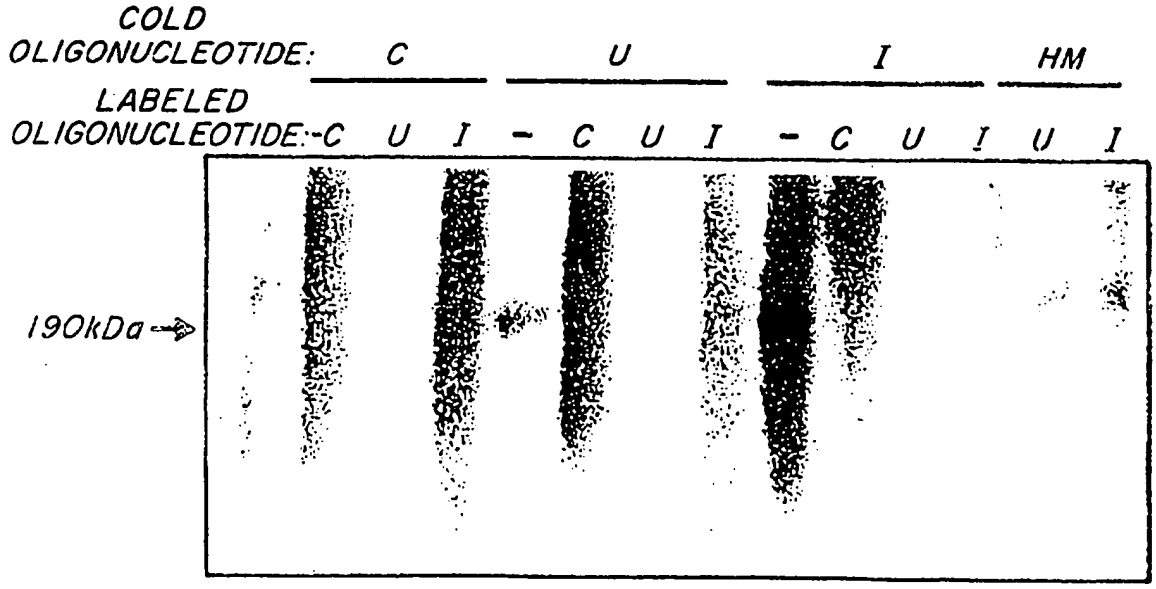

COLD
OLIGONUCLEOTIDE:
C
U
I
HM
LABELED
OLIGONUCLEOTIDE:-C U I - C U I - C U I U I
190kDa

FIG. 4B

TIME POST TREATMENT: 6 9 24 30
OLIGONUCLEOTIDE: C U I C U I C U I C U I

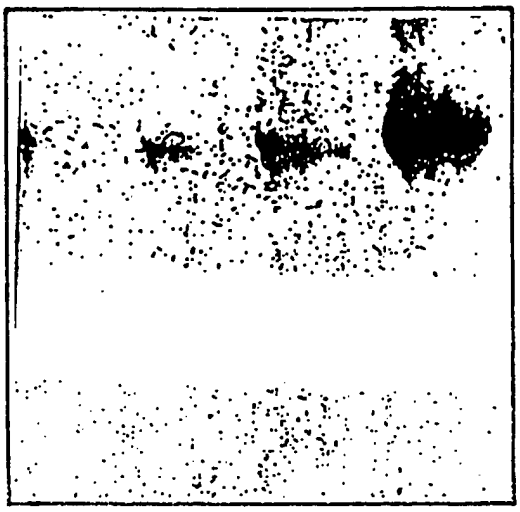


FIG. 5

FIG. 8

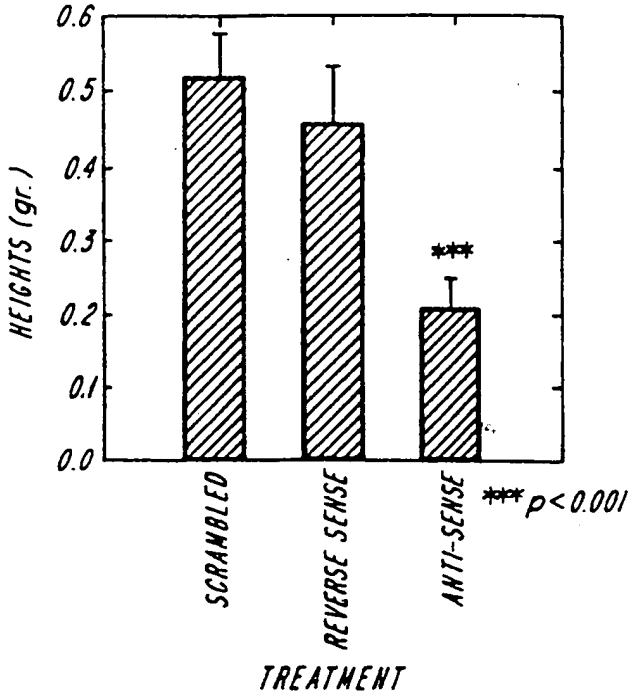

0.6
0.5
0.4
0.3
0.2
0.1
0.0
HEIGHTS (gr.)
***
SCRAMBLED
REVERSE SENSE
ANTI-SENSE
***p<0.001
TREATMENT

FIG. 7A

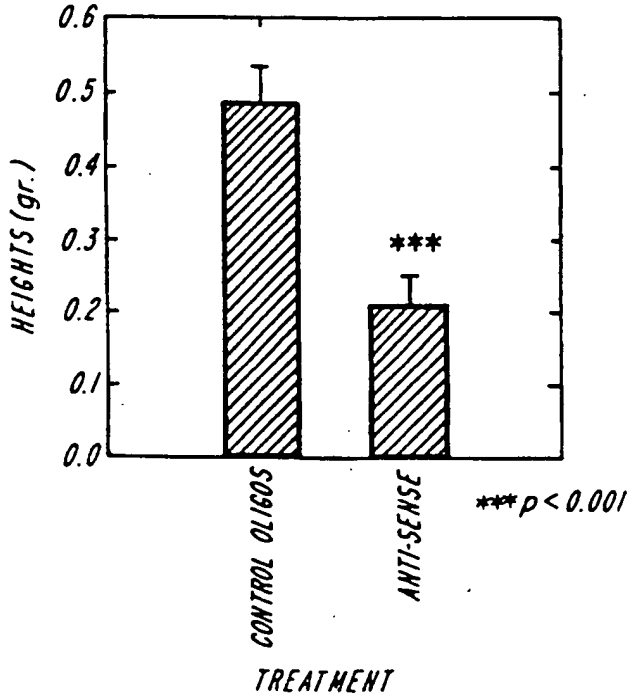

0.6
0.5
0.4
0.3
0.2
0.1
0.0
HEIGHTS (gr.)
***
CONTROL OLIGOS
ANTI-SENSE
***p<0.001
TREATMENT

FIG. 7B

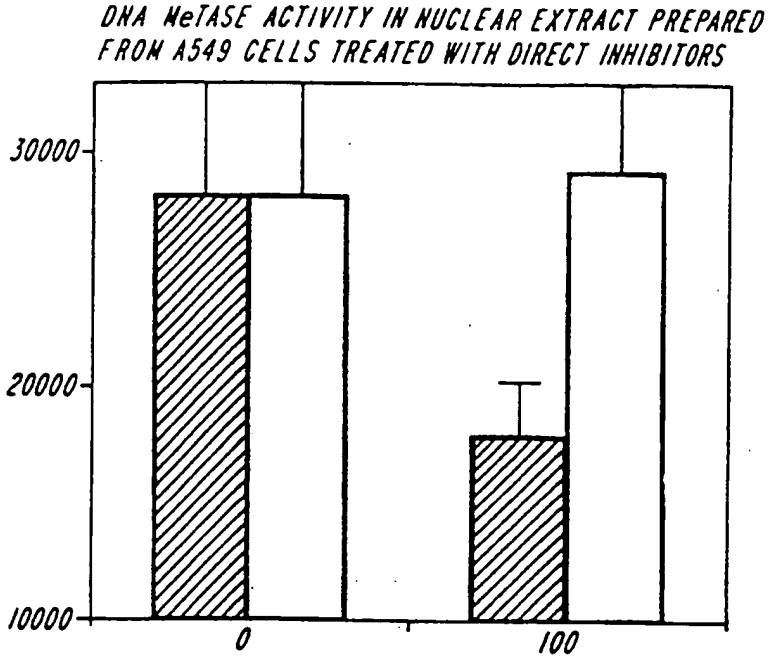


FIG. 9

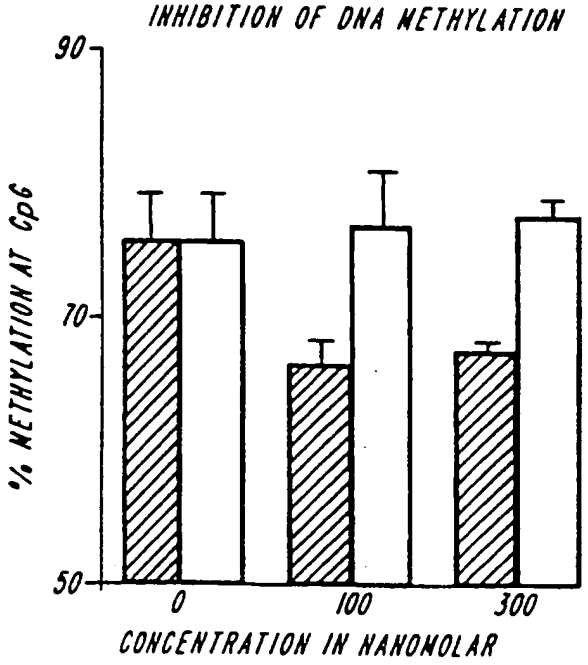


FIG. 10

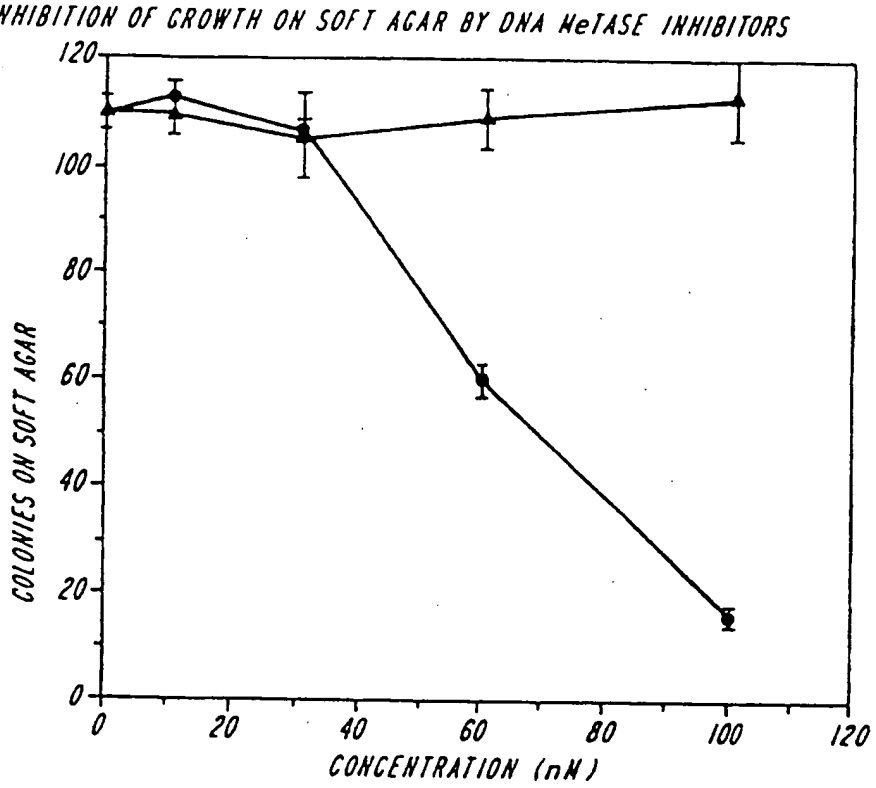

INHIBITION OF GROWTH ON SOFT AGAR BY DNA MeTASE INHIBITORS
COLONIES ON SOFT AGAR
0
20
40
60
80
100
120
CONCENTRATION (nM)

FIG. 11

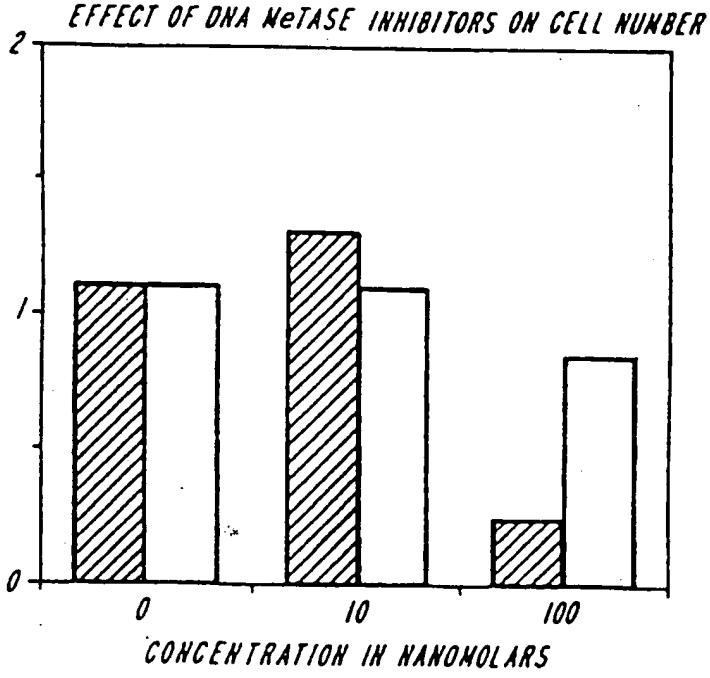

EFFECT OF DNA MeTASE INHIBITORS ON CELL NUMBER
2
1
0
0
10
100
CONCENTRATION IN NANOMOLARS

FIG. 12

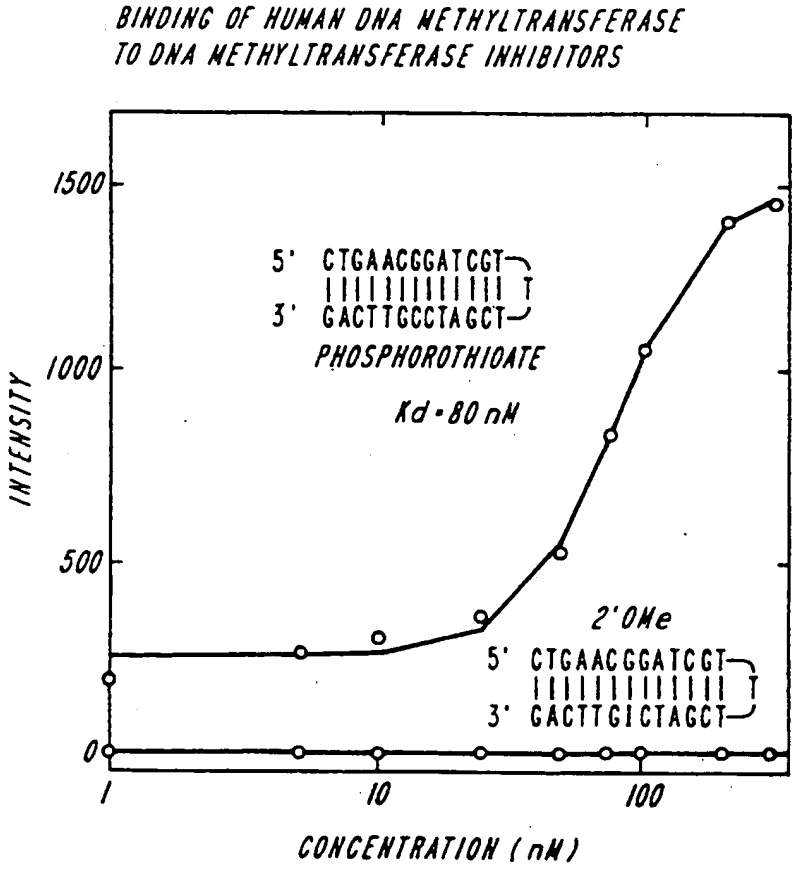


FIG. 13

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 9/10, C07H 21/00, C12N 15/11, C12Q 1/68, A61K 31/70

A3

(11) International Publication Number: WO 97/44346

(43) International Publication Date: 27 November 1997 (27.11.97)

(21) International Application Number: PCT/IB97/00879

(22) International Filing Date: 22 May 1997 (22.05.97)

(30) Priority Data:
08/653,954 22 May 1996 (22.05.96) US

(71) Applicant (*for all designated States except US*): McGILL UNIVERSITY [CA/CA]; 845 Sherbrooke Street West, Montréal, Québec H3A 1B1 (CA).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): SZYF, Moshe [CA/CA]; 5770 Melling, Cote St. Luc, Québec H4W 2Z7 (CA). BIGEY, Pascal [FR/FR]; 3, rue Docteur Chibert, F-63000 Clermont-Ferrand (FR).

(74) Agent: CÔTÉ, France; Swabey Ogilvy Renault, Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
5 February 1998 (05.02.98)

(54) Title: SPECIFIC INHIBITORS OF DNA METHYLTRANSFERASE ENZYME

(57) Abstract

The invention provides novel mechanism-based inhibitors of DNA methyltransferase enzyme, and diagnostic and therapeutic uses for the same. The novel inhibitors according to the invention form stable, noncovalent complexes with DNA methyltransferase enzyme in a manner which is independent of S-adenosylmethionine.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 97/00879

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N9/10 C07H21/00 C12N15/11 C12Q1/68 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07H C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 06985 A (HOPE CITY) 30 April 1992 see page 4, line 2 - page 5, line 16 see claims 1-6 ---	1-10
X	WO 95 15378 A (HYBRIDON INC ;UNIV MCGILL (CA); VON HOFE ERIC (US); SZYF MOSHE (CA) 8 June 1995 cited in the application see the whole document ---	1,3-5, 7-10
A	WO 95 15373 A (UNIV MCGILL ;SZYF MOSHE (CA)) 8 June 1995 see page 7, line 20 - line 34 ---	1
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
1 December 1997	15. 12. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Scott, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 97/00879

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FRIEDMAN S ET AL: "BINDING OF THE ECORII METHYLTRANSFERASE TO 5-FLUOROCYTOSINE-CONTAINING DNA. ISOLATION OF A BOUND PEPTIDE" NUCLEIC ACIDS RESEARCH, vol. 20, no. 12, 25 June 1992, pages 3241-3248, XP000644334 see abstract ---	1
A	KIM S C ET AL: "A NOVEL GENE-FUSING VECTOR: CONSTRUCTION OF A 5'-GGMCC-SPECIFIC CHIMERIC METHYLTRANSFERASE, M.BSPRI/M-BSURI" GENE, vol. 100, no. 1, 1991, pages 45-50, XP000644333 see abstract ---	1
A	A.TIMINSKAS ET AL.: "Sequence Motifs Characteristic for DNA (Cytosine-N4) and DNA (Adenine-N6) Methyltransferases. Classification of all DNA Methyltransferases." GENE, vol. 157, 1995, pages 3-11, XP002048745 see the whole document -----	1

1

INTERNATIONAL SEARCH REPORT

Int ıtional application No.
PCT/IB 97/00879

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 8 and 10 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 97/00879

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9206985 A	30-04-92	AU 648851 B	05-05-94
		AU 8953891 A	20-05-92
		CA 2072083 A	24-04-92
		EP 0506944 A	07-10-92
		US 5503975 A	02-04-96
WO 9515378 A	08-06-95	US 5578716 A	26-11-96
		AU 1296195 A	19-06-95
		EP 0731835 A	18-09-96
WO 9515373 A	08-06-95	CA 2110213 A	31-05-95
		AU 1061395 A	19-06-95
		CA 2177031 A	08-06-95
		EP 0734436 A	02-10-96
		JP 9506253 T	24-06-97